

Endothelial dysfunction in cystic fibrosis: Role of oxidative stress

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Running Title: Oxidative stress and vascular endothelial function in CF

1 **ABSTRACT**

2 Oxidative stress and vascular endothelial dysfunction are established
3 characteristics of cystic fibrosis (CF). Oxidative stress may contribute to vascular
4 dysfunction via inhibition of nitric oxide (NO) bioavailability. **Purpose:** To determine if
5 ingestion of an single antioxidant cocktail (AOC) improves vascular endothelial function
6 in patients with CF. **Methods:** In 18 patients with CF (age 8-39 y), brachial artery flow-
7 mediated dilation (FMD) was assessed using Doppler ultrasound prior to and two hours
8 following either an AOC (n=18; 1000 mg vitamin C, 600 IU vitamin E, and 600 mg α -lipoic
9 acid) or placebo (n=9). In a subgroup of patients (n=9), changes in serum concentrations
10 of α -tocopherol and lipid hydroperoxide (LOOH) were assessed following AOC and
11 placebo. **Results:** A significant ($p=0.032$) increase in FMD was observed following AOC
12 ($\Delta 1.9 \pm 3.3\%$), compared to no change following placebo ($\Delta -0.8 \pm 1.9\%$). Moreover,
13 compared with placebo, AOC prevented the decrease in α -tocopherol ($\Delta 0.48 \pm 2.91$ vs. -
14 $1.98 \pm 2.32 \mu\text{M}$, $p=0.024$) and tended to decrease LOOH ($\Delta -0.2 \pm 0.1$ vs. $0.1 \pm 0.1 \mu\text{M}$,
15 $p=0.063$). **Conclusions:** These data demonstrate that ingestion of an antioxidant cocktail
16 can improve vascular endothelial function and improve oxidative stress in patients with
17 CF, providing evidence that oxidative stress is a key contributor to vascular endothelial
18 dysfunction in CF.

19 INTRODUCTION

20 Cystic Fibrosis (CF) is the most prevalent autosomal recessive genetic disease in
21 North America. While the shortened life expectancy accompanying the disease can most
22 often be attributed to pulmonary infection (Cantin 1995), patients with CF also suffer from
23 a variety of systemic complications including dysfunction of the gastrointestinal, immune,
24 endocrine, and musculoskeletal systems (Gruet, Troosters, and Verges 2017; Plant et al.
25 2013).

26 The flow-mediated dilation (FMD) technique is a widely used, non-invasive
27 bioassay of conduit vessel endothelial function (Celermajer et al. 1992; Gori et al. 2011;
28 Uehata et al. 1997) and nitric oxide (NO) bioavailability (Green 2005). Our group has
29 recently provided evidence of both microvascular and conduit artery endothelial
30 dysfunction in patients with CF (Poore et al. 2013; Rodriguez-Miguel et al. 2016);
31 however, the mechanisms that contribute to vascular endothelial dysfunction in this
32 population have yet to be elucidated.

33 Considerable evidence indicates that systemic oxidative stress is a feature of CF
34 (Brown and Kelly 1994; Brown et al. 1996; Coates et al. 1980; Lezo et al. 2013; Montuschi
35 et al. 1999; Van Der Vliet et al. 1996; Wood et al. 2001) and may contribute to the
36 reduction in NO bioavailability and subsequent endothelial dysfunction (Zalba et al. 2001).
37 In CF, this imbalance between free radical production and neutralization of radicals by
38 antioxidants arises due to the combined effects of persistently elevated immune activation
39 (Galli et al. 2012; Wood et al. 2001) and both dietary deficiency and malabsorption of
40 exogenous antioxidants (Brown et al. 1996; Galli et al. 2012; Wood et al. 2001).
41 Administration of oral antioxidants has been demonstrated to temporarily reduce oxidative

42 stress and improve vascular function in other populations (Ryan A Harris et al. 2009; Wray
43 et al. 2012; Sánchez-Moreno et al. 2004; Ives et al. 2014); however, the role of oxidative
44 stress in vascular dysfunction in patients with CF is unknown. Therefore, this study
45 sought to test the hypothesis that a single dose of an antioxidant cocktail would reduce
46 oxidative stress and improve vascular endothelial function, whereas no change would be
47 observed following a placebo condition.

48

49 **MATERIALS AND METHODS**

50 ***Participants***

51 **Figure 1** illustrates the recruitment and testing process for participants in this
52 study. Based on the efficacy of the antioxidant cocktail (AOC) in other clinical populations
53 (Ives et al. 2014; Wray et al. 2012), a proof of concept efficacy trial of the AOC was
54 conducted in 9 patients during one visit. Following this initial study, 9 additional patients
55 with CF were recruited to take part in a double blind, randomized, placebo-controlled,
56 crossover trial where patients received the AOC (CF-AOC) and placebo (CF-PLC) in
57 randomized order on separate experimental visits. Of our patient population, 50% were
58 homozygous F508del, 22% were F508del/G551D, 22% were heterozygous with one copy
59 of F508del, and 11% were heterozygous without f508del. Only the four patients with
60 gating mutations were on modulator therapy (ivacaftor) and had been taking it for at least
61 3 months prior to testing. To further examine the impact of the AOC on oxidative stress,
62 circulating markers of oxidative stress balance were determined prior to and 2 hours
63 following AOC or placebo treatment. 18 demographically matched (age, sex, height,
64 weight, and BMI) healthy controls were recruited to provide a reference standard of

65 vascular function and to determine the efficacy of the treatment response in patients with
66 CF. The control group did not undergo any treatment, nor were any of the pre-post
67 treatment biomarkers evaluated.

68 All patients were enrolled if they had a clinical diagnosis of CF based on positive
69 sweat tests and genotype analysis. Participants were excluded if they 1) had a forced
70 expiratory volume in one second (FEV_1) < 50% of predicted, 2) had a resting oxygen
71 saturation (SpO_2) < 85%, 3) self-reported to be a smoker, 4) were diagnosed with
72 pulmonary hypertension, 5) were pregnant or nursing at the time of the investigation, 6)
73 had a clinical diagnosis of cardiovascular disease, hypertension, or CF related diabetes,
74 or 7) were prescribed any vaso-active medications (i.e. nitrates, beta blockers, ACE
75 inhibitors, etc.). All participants and parents of children provided written and verbal
76 consent/assent prior to participation. All study protocols were approved by the Institutional
77 Review Board at Augusta University. This study was registered to the clinicaltrials.gov
78 website (#NCT01772758).

79

80 ***Experimental Design***

81 All participants reported to the Laboratory of Integrated Vascular and Exercise
82 Physiology (LIVEP) at the Georgia Prevention Institute for a preliminary visit that
83 consisted of the informed consent process, body composition assessments, and a
84 baseline pulmonary function test (PFT). For each of the experimental visits, participants
85 reported to the LIVEP in the morning following an overnight fast, and having abstained
86 from moderate to vigorous physical activity for 24 hours prior to investigation. Patients
87 were instructed to adhere to the timing of their daily pulmonary therapy and come to the

88 lab following their morning airway clearance and inhaled medicines. Upon arrival,
89 baseline assessments of PFT and flow-mediated dilation (FMD) were performed and a
90 venous blood sample was obtained. Patients were then given either an oral AOC (CF-
91 AOX; 1000 mg vitamin C, 600 IU vitamin E, and 600 mg α -lipoic acid) or a visually similar
92 cocktail of placebo pills (CF-PLC; sucrose or galactose). Following ingestion of treatment,
93 patients rested quietly for two hours and a post-treatment FMD was performed.

94

95 ***Participant Characteristics and Clinical Laboratory Values***

96 Height and weight were determined using a stadiometer and standard platform
97 scale (CN20, DETECTO[®], Webb City, MO) and used for calculations of body mass index
98 (BMI). Total body fat, fat-mass, and fat-free mass were determined using dual energy X-
99 ray absorptiometry (QDR-4500W; Hologic, Waltham, MA) and resting systolic and
100 diastolic blood pressures were evaluated using established protocols (Kapuku et al.
101 1999). Resting oxygen saturation was obtained using an Onyx II fingertip sensor (Nonin
102 Medical, Plymouth, MN). Fasting concentrations of total cholesterol (TC), high-density
103 lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides (TG), and glucose were
104 obtained using a Cholestech LDX point of care analyzer (Alere Inc., Scarborough, ME).
105 Hemoglobin and hematocrit were determined using a HemoPoint H2 analyzer (Stanbio
106 Laboratories). Concentrations of high-sensitivity C-reactive protein (hsCRP) were
107 obtained from standard core laboratory techniques (Laboratory Corporation of America
108 Holdings, Burlington, NC).

109

110

111 ***Biomarkers of Oxidative Stress and Lipid Soluble Antioxidants***

112 Markers of oxidative stress balance were determined prior to and following the
113 administration of the AOC and PLC. Plasma concentrations of 8-isoprostane (Cayman
114 Chemical, Ann Arbor, MI) and nitrotyrosine (Cell Biolabs, Inc., San Diego, CA) were
115 determined via colorimetric assay following the manufacturer's instructions. Total serum
116 hydroperoxide (LOOH) concentrations were determined by the ferrous oxidation-xylene
117 orange (FOX1) assay (Wolff 1994) using a protocol previously described by our group
118 (Medlow et al. 2015).

119 Serum α -tocopherol, γ -tocopherol, retinol, and lycopene were determined using
120 high performance liquid chromatography (HPLC) as previously described in a protocol by
121 our group (Medlow et al. 2015). Data were analyzed by Empower analytical software
122 (Waters, Ireland).

123

124 ***Pulmonary Function Testing (PFT)***

125 An assessment of pulmonary function was performed using the EasyOne Pro®
126 LAB system (ndd Medical Technologies, Andover, MA) to determine forced vital capacity
127 (FVC), FEV₁ (L), FEV₁ (% predicted), FEV₁/FVC, and forced expiratory flow at 25-75%
128 (FEF₂₅₋₇₅) in all participants according to the American Thoracic Society standards
129 (Kellogg et al. 1995). Briefly, following the American Thoracic Associations
130 recommendations (Society 1995), a minimum of three reproducible trials were completed
131 by each participant and the best of three acceptable forced expiratory maneuvers was
132 used for analysis. The European Respiratory Society Global Lung Function Initiative

133 spirometric reference standards were used to determine the percentage predicted data
134 set (Quanjer et al. 2012).

135

136 ***Flow-Mediated Dilation (FMD) and Shear Rate***

137 Brachial artery FMD was determined using Doppler ultrasound (Logiq 7, GE
138 Medical Systems, Milwaukee, WI) performed in accordance with published guidelines (R.
139 A. Harris et al. 2010) and methodology previously described by our group (Poore et al.
140 2013; R. A. Harris et al. 2012). Briefly, simultaneous B-mode and blood velocity profiles
141 of the brachial artery were evaluated by ultrasound imaging using a 12-MHz linear
142 transducer. After acquisition of baseline values, a forearm occlusion cuff placed
143 immediately distal to the medial epicondyle, was rapidly inflated to 250 mm Hg for 5 min
144 (E-20 rapid cuff inflator, Hokanson) to induce arterial occlusion and then deflated to
145 induce reactive hyperemia of the brachial artery. R-wave gating (AccuSync 72, AccuSync
146 Medical Research, Milford, CT) was used to capture end-diastolic arterial diameters for
147 automated offline analysis of brachial artery vasodilation (Medical Imaging Applications,
148 Coralville, IA). The greatest 5-s diameter average after cuff release was used as the peak
149 response. FMD was expressed as the percent increase in peak diameter from baseline
150 diameter and also relative to shear rate (FMD/shear).

151 Cumulative shear rate (area under the curve [AUC, s⁻¹]) and FMD/shear were
152 determined as previously described by our group (R. A. Harris et al. 2012; Poore et al.
153 2013). Absolute change in diameter, peak diameter, and time to peak dilation were
154 calculated and reported according to published guidelines and recommendations

155 (Thijssen et al. 2011) to provide a comprehensive assessment of vascular endothelial
156 function.

157

158 ***Statistical Analyses***

159 All analyses were performed using SPSS version 24 (IBM Corporation, Somers,
160 NY). Descriptive statistics were generated and range as well as normality checks were
161 performed. Independent t-tests were performed to identify differences in demographics,
162 clinical laboratory markers, and pulmonary function parameters between patients with CF
163 and healthy controls. Comparisons of baseline (pre-treatment) parameters of the FMD
164 test between CF-AOC and CF-PLC groups were performed using independent t-tests. A
165 two-way (group by time) ANOVA was used to test for pre- to post-treatment differences
166 in parameters of the FMD test and markers of oxidative stress between AOC and PLC.
167 Covariates related to disease severity (FEV₁ [% predicted] and HbA1c as an index of
168 glycemic control) were included as covariates in the regression model where appropriate.
169 Effect sizes (partial eta squared [η_p^2]) are reported for the interaction terms of the ANOVA,
170 where values of 0.01, 0.06, and 0.14 correspond to small, medium, and large effects,
171 respectively (Cohen 1988). Values are presented as mean \pm SD unless otherwise noted.
172 An alpha <0.05 was considered statistically significant for all analyses.

173

174 **RESULTS**

175 ***Participant Characteristics, Clinical Laboratory Values, and Pulmonary Function***

176 Baseline characteristics, clinical laboratory values, and indices of pulmonary
177 function for patients with CF and healthy controls are presented in **Table 1**. There were

178 no differences in demographic or anthropometric characteristics between patients and
179 controls; however, patients exhibited significantly lower ($p<0.05$) TC, and HDL, and
180 significantly higher ($p=0.003$) hsCRP compared to controls. There were no differences in
181 FVC between groups; however, patients had significantly lower absolute FEV₁, FEV₁ (%
182 predicted), FEV₁/FVC, and FEF₂₅₋₇₅ versus controls (all $p<0.05$). In addition, while resting
183 SpO₂ was at a normal level in patients (98%), it was significantly lower compared with
184 controls ($p=0.005$).

185

186 ***Flow-Mediated Dilatation***

187 **Figure 2** illustrates a significant improvement ($p=0.032$, $\eta_p^2=0.170$) in FMD
188 following the AOC, whereas no change was observed following placebo. Additional
189 parameters of the FMD test are presented in **Table 2**. There was a significant increase
190 ($p=0.004$) in FMD normalized for shear rate (FMD/shear) and decrease in time to peak
191 dilation (TTP; $p=0.011$) in CF-AOC and CF-PLC, but changes were not different between
192 groups ($p=0.137$, $\eta_p^2=0.086$ and $p=0.288$, $\eta_p^2=0.045$, respectively). While the change in
193 absolute diameter was significantly greater ($p=0.023$, $\eta_p^2=0.189$) in CF-AOC versus CF-
194 PLC, changes in baseline diameter ($p=0.622$, $\eta_p^2=0.010$), peak diameter ($p=0.115$,
195 $\eta_p^2=0.096$), and shear rate ($p=0.820$, $\eta_p^2=0.002$) were not different between groups.

196 In addition to the CF patient data, FMD data from demographically-matched,
197 healthy participants are also presented in **Table 2** as a control reference of normal
198 vascular endothelial function. While pre-treatment FMD (%) was not significantly different
199 in CF-AOC or CF-PLC versus controls ($p=0.101$ and $p=0.590$, respectively), pre-
200 treatment FMD/shear was significantly lower ($p=0.010$) in CF-AOC versus controls. This

201 deficit, however, was improved following the AOC treatment, leading to restoration in both
202 post-treatment FMD and FMD FMD/shear compared to controls ($p=0.660$).

203 There were no differences between CF-AOC or CF-PLC and controls in pre-
204 treatment baseline diameter ($p=0.463$ and $p=0.077$, respectively), peak diameter
205 ($p=0.688$ and $p=0.126$), or absolute change in diameter ($p=0.434$ and $p=0.753$); however,
206 TTP was lower in controls versus both CF-AOC ($p=0.008$) and CF-PLC ($p=0.024$).

207

208 ***Biomarkers of Oxidative Stress and Lipid Soluble Antioxidants***

209 Baseline (pre-treatment) levels of α -tocopherol (21.7 ± 14.4 vs. 21.3 ± 16.5 μM ,
210 $p=0.788$), lycopene (0.06 ± 0.06 vs. 0.05 ± 0.09 μM , $p=0.610$), and LOOH (0.74 ± 0.11
211 vs. 0.87 ± 0.22 μM , $p=0.164$) were not different between PLC and AOC. **Figure 3**
212 illustrates the change in oxidative stress balance following the AOC or PLC. Specifically,
213 reductions in α -tocopherol ($p=0.024$, $\eta_p^2=0.54$) and lycopene ($p=0.014$, $\eta_p^2=0.60$) were
214 significantly attenuated following AOC compared with PLC while controlling for HbA1c.
215 While not significant, LOOH tended to decrease ($p=0.063$, $\eta_p^2=0.33$) following the AOC
216 versus PLC. Additional systemic markers of oxidative stress and lipid soluble antioxidants
217 are presented in **Table 3**. AOC treatment changes in 8-isoprostane ($p=0.815$, $\eta_p^2=0.01$),
218 nitrotyrosine ($p=0.820$, $\eta_p^2=0.01$), γ -tocophorol ($p=0.220$, $\eta_p^2=0.21$), and retinol ($p=0.121$,
219 $\eta_p^2=0.31$) were all similar to PLC.

220

221 **DISCUSSION**

222 Cystic fibrosis is associated with a variety of systemic complications including
223 vascular endothelial dysfunction (Poore et al. 2013; Rodriguez-Miguel et al. 2016).

224 However, the mechanism(s) contributing to this dysfunction in CF have yet to be
225 elucidated. To the best of our knowledge, this is the first study to investigate oxidative
226 stress as a potential mechanism that contributes to vascular endothelial dysfunction in
227 CF. Findings from the present study support our hypothesis that a single dose of an AOC
228 elicits a significant improvement in vascular endothelial function compared to no change
229 with placebo (**Figure 2**). In addition, AOC treatment significantly prevented the reduction
230 in circulating concentrations of α -tocopherol and tended to decrease LOOH compared to
231 placebo (**Figure 3**). Together, these findings provide strong mechanistic evidence that
232 oxidative stress contributes to vascular dysfunction in patients with CF.

233 Recently, our group provided the first evidence of both conduit- and micro-
234 vascular endothelial dysfunction in young patients with CF (Rodriguez-Miguel et al.
235 2016; Poore et al. 2013). The FMD test is not only reproducible in patients with CF
236 (Derella et al. 2019), it allows for non-invasive assessment of vascular endothelial
237 function (Celermajer et al. 1992; Gori et al. 2011; Uehata et al. 1997) and, importantly,
238 nitric oxide (NO) bioavailability (Green 2005). NO-dependent vasodilation is perhaps the
239 most important signaling function of the endothelium due to the protective effect against
240 the development of atherosclerosis (Knowles and Moncada 1994). Oxidative stress, an
241 established characteristic of CF (Brown and Kelly 1994; Brown et al. 1996; Coates et al.
242 1980; Lezo et al. 2013; Montuschi et al. 1999; Van Der Vliet et al. 1996; Wood et al.
243 2001), can negatively impact endothelial function as NO rapidly reacts with superoxide
244 (Szabo, Ischiropoulos, and Radi 2007; Pacher, Beckman, and Liaudet 2007) and reduces
245 NO bioavailability (Zalba et al. 2001).

246 The etiology of oxidative stress in CF is related to both pulmonary and non-pulmonary
247 manifestations of the disease. First, CF directly causes chronic pulmonary infection that
248 not only contributes to a persistently elevated pro-inflammatory immune response, it also
249 results in overproduction of reactive oxygen species (ROS) by activated leukocytes (Galli
250 et al. 2012; Wood et al. 2001). Although basal inflammation (i.e. CRP) was higher in
251 patients compared to controls (Table 1), inflammatory biomarkers were not assessed as
252 we did not anticipate any changes in systemic inflammation following a single AOC.
253 Second, the dysfunctional cystic fibrosis transmembrane regulator (CFTR) gene
254 contributes to pancreatic insufficiency and nutrient malabsorption (Singh and
255 Schwarzenberg 2017) which leads to diminished secretion of pancreatic enzymes,
256 dysfunctional lipid digestion, and ultimately, reduced absorption of fat-soluble vitamins.
257 Indeed, several of these essential vitamins serve as antioxidants (e.g., vitamins A and E)
258 and their impaired absorption likely contributes to oxidative stress. For this reason, many
259 patients with CF are prescribed daily fat-soluble vitamins (e.g., AquADEK); however, the
260 AOC used in the present study may have even greater therapeutic potential for several
261 reasons. First, α -tocopherol, the main lipid chain breaking antioxidant, is maintained only
262 in the presence of ascorbic acid (Scarpa et al. 1984), and rapid reactions between the
263 two encourages recycling of α -tocopherol. Further, α -lipoic acid, a powerful dual phase
264 (aqueous and lipid) antioxidant (Wollin and Jones 2003), aids in the reduction of
265 dehydroascorbic acid to ascorbic acid (Xu and Wells 1996), highlighting its recycling
266 ability and involvement in complex antioxidant networks. Thus, the combination of
267 antioxidants used in our AOC work synergistically to combat oxidative stress. Indeed,
268 utilizing a placebo controlled within-patient experimental design, our data indicate that the

269 AOC significantly prevented the reduction in α -tocopherol and tended to reduce indices
270 of oxidative stress (**Figure 3**). In addition, these data support the ability of the CF gut to
271 absorb the AOC into circulation. Encouragingly, FMD following the AOC in both children
272 and adults was restored to a value similar to that of healthy controls, possibly due to
273 improved ROS buffering capacity and an increase in NO bioavailability (**Table 2**).
274 Although outside the scope of the present investigation and unlikely to impact the findings
275 following a single experimental treatment, we cannot rule out the potential effects of CFTR
276 genotype and modulator therapies acting on the vasculature. The CFTR gene is
277 expressed on endothelial cells and may impact vascular reactivity independent of
278 oxidative stress balance. Further studies are certainly warranted to clarify the potential
279 influence of CFTR therapies on vascular endothelial function in CF.

280 Taken together, these observations provide compelling evidence to support the
281 role of oxidative stress as a key contributor to vascular endothelial dysfunction in CF. Our
282 findings suggest that an oral AOC is capable of reducing oxidative stress and may provide
283 therapeutic benefit for patients with CF. Indeed, the present findings warrant further
284 investigation to determine the impact of extended (i.e., >6 months) antioxidant treatment
285 on oxidative stress and vascular function in this patient population.

286

287 **Clinical Significance**

288 The development of cardiovascular disease (CVD) is closely tied to endothelial
289 dysfunction (Vanhouste et al. 2009) and a 1% decrease in FMD is associated with an ~8%
290 increase in risk of future cardiovascular events (Inaba, Chen, and Bergmann 2010). In
291 the present study, the 1.9% increase in FMD observed following the AOC treatment

292 translates to a ~15% risk reduction for future cardiovascular events. Beyond the potential
293 impact antioxidant treatments may have on CVD risk reduction in CF, previous work from
294 our group has implicated endothelial dysfunction as a contributor to exercise capacity
295 (Poore et al. 2013) and exercise blood flow regulation (Tucker et al. 2018); key areas of
296 concern for patients with CF given that exercise intolerance is an independent predictor
297 of mortality in this population (Nixon et al. 1992; Pianosi, Leblanc, and Almudevar 2005).
298 Thus, the therapeutic potential of antioxidant treatments in CF to improve endothelial
299 function may have far-reaching clinical implications and warrants further investigation.

300

301 **Conclusions**

302 This is the first known study to investigate oxidative stress as a potential
303 mechanism that contributes to vascular endothelial dysfunction in patients with CF.
304 Importantly, ingestion of a single oral antioxidant cocktail treatment in patients not only
305 improved FMD, but restored endothelial function to the value of healthy controls.
306 Collectively, the improvement in oxidative stress balance coupled with the improved FMD
307 following the antioxidant cocktail treatment indicate that oxidative stress is an important
308 contributor to endothelial dysfunction in CF. Future studies are needed to determine if
309 chronic antioxidant administration can lead to sustained improvements in endothelial
310 function in patients with CF.

311

312 **Data Availability**

313 The data used to support the findings of this study are available from the corresponding
314 author upon request.

315

316 **Conflicts of Interest**

317 None to declare.

318

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481 **FIGURE CAPTIONS**

482

483 **Figure 1.** Schematic illustrating the recruitment/enrollment process and overall
484 experimental design. Flow-mediated dilation (FMD) was assessed in healthy controls
485 (n=18) and in patients with CF (n=18) following an antioxidant cocktail (AOC). In a
486 subgroup of patients with CF (n=9), measures of oxidative stress balance were
487 assessed following ingestion of the AOC and a placebo condition.

488

489 **Figure 2.** Changes in flow-mediated dilation (FMD) in patients with CF following either
490 the AOC treatment (CF-AOC; n=18) or placebo (CF-PLC; n=9). *Significantly greater
491 versus CF-PLC (p=0.032). Values are presented as mean \pm SEM.

492

493 **Figure 3.** Changes in plasma levels of antioxidants (panels A and B) and lipid
494 hydroperoxide (LOOH, panel C) in patients with CF in the AOC treatment and placebo
495 (PLC) condition (n=9). *significant difference between treatments when controlling for
496 HbA1c as an index of disease severity (p<0.05).

Table 1. Participant characteristics, clinical laboratory markers, and pulmonary function in patients with CF and controls.

Variable	CF	Controls	P value
N	18	18	
Sex (M/F)	8/10	8/10	
Age (y)	18.8 ± 9.4	15.7 ± 5.2	0.227
Height (cm)	158 ± 15	163 ± 15	0.337
Weight (kg)	53.4 ± 15.9	52.5 ± 16.5	0.868
BMI (kg/m ²)	20.8 ± 3.3	19.3 ± 3.9	0.225
Body Fat (%)	22.0 ± 6.3	22.3 ± 8.2	0.902
SBP (mmHg)	108 ± 12	108 ± 16	0.915
DBP (mmHg)	60 ± 7	63 ± 8	0.133
Resting SpO ₂ (%)	97.9 ± 1.5	99.0 ± 0.6	0.005
Clinical Laboratory Markers			
TC (mg/dL)	127 ± 22	148 ± 26	0.009
HDL (mg/dL)	42 ± 12	55 ± 11	0.002
LDL (mg/dL)	66 ± 16	73 ± 36	0.508
Triglycerides (mg/dL)	88 ± 31	73 ± 27	0.144
Glucose (mg/dL)	86 ± 14	84 ± 9	0.658
TC:HDL	3.2 ± 0.7	2.8 ± 0.6	0.075
hsCRP	2.31 ± 2.33	0.51 ± 0.33	0.003
Pulmonary Function			
FVC (L)	3.66 ± 1.25	4.10 ± 1.28	0.291
FEV ₁ (L)	2.78 ± 1.00	3.53 ± 0.98	0.025
FEV ₁ (% predicted)	88.0 ± 18.1	104.4 ± 9.6	0.002
FEV ₁ /FVC (%)	75.5 ± 9.2	87.6 ± 7.3	<0.001
FEF ₂₅₋₇₅ (L/s)	2.47 ± 1.23	4.06 ± 1.27	<0.001

Values are mean ± SD. BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; SpO₂, oxygen saturation; TC = total cholesterol; HDL = high density lipoprotein; LDL = low density lipoprotein; hsCRP = high sensitivity C-reactive protein; FVC = forced vital capacity; FEV₁ = forced expiratory volume in 1 second; FEF₂₅₋₇₅ = forced expiratory flow.

Table 2. Parameters of the FMD test in patients with CF completing the AOC treatment (CF-AOC; n=18), placebo condition (CF-PLC; n=9), or healthy controls (n=18).

Variable	CF-AOC		CF-PLC		Controls
	Pre	Post	Pre	Post	
Baseline diameter (cm)	0.306 ± 0.055	0.302 ± 0.053	0.328 ± 0.049	0.325 ± 0.051	0.294 ± 0.043
Peak diameter (cm)	0.323 ± 0.056	0.324 ± 0.054	0.348 ± 0.048	0.344 ± 0.053	0.315 ± 0.052
FMD absolute change (cm)	0.017 ± 0.008	0.022 ± 0.011*	0.021 ± 0.012	0.018 ± 0.008	0.022 ± 0.012
Shear rate (s ⁻¹ , AUC)	58,273 ± 29,735	51,089 ± 24,393	52,527 ± 25,003	43,242 ± 30,749	45,737 ± 10,735
FMD/Shear (% / s ⁻¹ , AUC)	0.11 ± 0.06	0.15 ± 0.06	0.14 ± 0.09	0.16 ± 0.08	0.16 ± 0.06 [†]
Time to peak (s)	58.1 ± 28.1	45.3 ± 19.9	70.8 ± 35.3	41.4 ± 16.4	38.1 ± 10.1 ^{††}

Values are mean ± SD. FMD = flow-mediated dilation. *significant pre- to post-treatment change versus CF-PLC (p<0.05); [†]significant difference versus Pre in CF-AOC (p<0.05); ^{††}significant difference versus Pre in CF-PLC (p=0.024).

Table 3. Biomarkers of oxidative stress and lipid soluble antioxidants in patients with CF following the AOC treatment and placebo (PLC) condition (n=9).

Variable	CF-AOC			CF-PLC		
	Pre	Post	Change	Pre	Post	Change
8-isoprostane (pg/ml)	9.9 ± 3.8	11.4 ± 4.5	1.5 ± 1.6	10.9 ± 6.8	11.6 ± 7.5	0.8 ± 1.5
Nitrotyrosine (nM)	172.7 ± 69.4	180.1 ± 72.3	7.4 ± 25.3	200.1 ± 84.8	215.5 ± 52.2	15.4 ± 84.6
γ-tocopherol (μM)	2.16 ± 1.16	1.97 ± 0.78	-0.19 ± 1.34	2.12 ± 0.95	1.58 ± 0.81	-0.54 ± 0.84
Retinol (μM)	2.36 ± 0.83	2.20 ± 0.72	-0.16 ± 0.93	2.22 ± 0.99	2.10 ± 0.94	-0.12 ± 1.11

Values are mean ± SD.